PB86-110681

Use of Bioassays in Determining the Toxicity of Sediment to Benthic Organisms

(U.S.) Corvallis Environmental Research Lab.
Newport, OR

Sep 85

U.S. Department of Commerce
National Technical Information Service
NTIS

PB86-110681

EPA/600/D-85/221
September 1985

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USE OF BIOASSAYS IN DETERMINING THE TOXICITY OF SEDIMENT TO BENTHIC ORGANISMS

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USE OF BIOASSAYS IN DETERMINING THE TOXICITY OF SEDIMENT TO BENTHIC AQUATIC ORGANISMS

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ABSTRACT

Sediment bioassay methods range in complexity from short-term acute tests of the effects of individual contaminants on single species, to long-term tests of the effects of chemical mixtures on the function of benthic mesocosms. The phoxocephalid amphipod bioassay developed in our laboratory, as well as other marine and freshwater sediment bioassays, can be used to determine the spatial distribution of sediment toxicity along pollution gradients, to identify "hot spots" in contaminated areas, or to determine the toxicity of materials experimentally added to sediment. Sediment bioassays are being used successfully in regulatory programs. Research needs include field validation of bioassay results, bioavailability of sediment toxicants, toxicological interactions between sediment contaminants, and the ecological consequences of toxic sediment.

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1. INTRODUCTION

Sediments have often been called a cumulative sink or a major repository for persistent toxic substances in the aquatic environment (Chapman, 1985; Oschwald, 1972; Swartz and Lee, 1980). Contaminated sediments may contain depauperate benthic community assemblages even in areas where overlying waters appear "clean" (Laskowski-Hoke and Prater, 1984). Through the processes of diffusion, physical disruption of the sediments, or bioturbation and other biological processes, toxicants may be transferred from sediments to the overlying water column (Lee

and Swartz, 1980; Nalepa et al., 1980; Swartz and Lee, 1980). Toxic substances sorbed on sediment particulates may be ingested by sediment feeding benthic organisms, or may be dissolved in interstitial water and assimilated through respiration, ingestion, or other absorptive processes. Toxicants may thus directly affect benthic organisms or enter the food chain as these organisms are consumed.

Methods employed to determine the degree of contamination of sediments frequently involve chemical analyses of the sediments or studies on benthic community structure. However, with currently available chemical methods, it is costly and difficult to analyze for the hundreds of potentially toxic chemical compounds that may be present in sediments (Malins et al., 1984). Chemical analyses give little information on bioavailability of compounds, biological effects on benthic organisms, or effects of interactions among chemicals on the behavior of the chemicals in the sediment or in living organisms. Structural analyses of benthic communities, in addition to being time consuming and thereby limited in the number of samples which can be conveniently analyzed, may fail to take into account the inherent spatial and temporal variability of benthic ecosystems due to seasonal biological or physical processes.

Sediment bioassays are comparatively inexpensive and simple, and can provide rapid information on the degree of toxicity of potentially contaminated sediments to benthic organisms. Acute and chronic toxicity bioassays have been widely used

since the 1950's to evaluate the effects of wastes on organisms in receiving waters (Buikema et al., 1982) and to develop water quality criteria, but comparatively few toxicity bioassays have been developed for sediments. Because sediment bioassays can provide direct evidence of adverse biological effects occurring from exposure of organisms to toxicants in sediments, their use has been recommended in combination with other methodologies to assess the toxicity of contaminated sediment (Chapman, 1985; Chapman and Long, 1983; Long, 1983).

2. OVERVIEW OF SEDIMENT BIOASSAY METHODS

2.1. Marine

Since the EPA/COE Implementation Manual (EPA/COE, 1977) provided guidance for development of sediment bioassays to test the toxicity of dredged materials, a variety of procedures have been developed to evaluate sediment contamination in the marine environment (reviewed by Chapman, 1985 and Swartz, 1985). These range in complexity from short-term acute tests of the effects of individual contaminants on single species, to long-term determinations of the effects of chemical mixtures on the function of benthic microcosms. Sediments tested are usually solid phase, although sometimes suspended solids, elutriates or sediment extracts are examined. Amounts of sediments tested range from a few grams to over 800 liters, and systems may be static or flow-through. Test procedures

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1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA/600/D-85/221		PB86 170681/AS

4. TITLE AND SUBTITLE	5. REPORT DATE
USE OF BIOASSAYS IN DETERMINING THE TOXICITY OF SEDIMENT TO BENTHIC ORGANISMS	September 1985
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
J.O. Lamberson andR.C. Swartz	ERLN-N013
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
U.S.EPA, ERLN-Newport Hatfield Marine Science Center Newport, OR 97365	AARB1A
	11. CONTRACT/GRANT NO.
	NA
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Environmental Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Narragansett, RI 02882	Symposium Proceedings
	14. SPONSORING AGENCY CODE
	EPA/600/05

15. SUPPLEMENTARY NOTES

16. ABSTRACT Sediment bioassay methods range in complexity from short-term acute tests of the effects of individual contaminants on single species, to long-term tests of the effects of chemical mixtures on the function of benthic mesocosms. The phoxocephalid amphipod bioassay developed in our laboratory, as well as other marine and freshwater sediment bioassays, can be used to determine the spatial distribution of sediment toxicity along pollution gradients, to identify "hot spots" in contaminated areas, or to determine the toxicity of materials experimentally added to sediment. Sediment bioassays are being used successfully in regulatory programs. Research needs include field validation of bioassay results, bioavailability of sediment toxicants, toxicological interactions between sediment contaminants, and the ecological consequences of toxic sediment.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
RELEASE TO PUBLIC	Unclassified	33
	20. SECURITY CLASS *(This page)*	22. PRICE
	Unclassified	$9.95

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

involve organisms such as fish and a variety of benthic, epibenthic and pelagic invertebrates including nematodes, oligochaetes, polychaetes, pelecypods, crustacea, echinoderms and tunicates. Response criteria, ranging from acute mortality through changes in mesocosm structure and function, include sublethal physiological responses such as respiration, cellular function, and behavioral and developmental variations. Duration extends from ten minutes to over a year. Bioassay test procedures have been applied to materials collected from potentially contaminated field sites, or less frequently, to uncontaminated sediments "spiked" with specific chemicals. In our laboratory, acute, static bioassay procedures for marine sediments have been developed using the infaunal amphipod Rhepoxynius abronius (Swartz et al., 1985a) and have been extensively applied to both field-collected and laboratory-spiked sediments.

2.2. Fresh Water

Methods for determining toxicity of contaminated freshwater sediments have been reviewed by Chapman (1985) and Nebeker et al. (1984). Most research involving freshwater sediment bioassays to date has been concerned with methods development and field and laboratory studies on heavy metal contamination. Several bioassay methods have been employed to rank field sediments on the basis of toxicity to test organisms (LeBlanc

and Surprenant, 1985; Malueg et al., 1984b; Prater and Anderson, 1977a; Prater and Hoke, 1978; Samoiloff et al., 1983). Malueg et al. (1984a,b) applied acute sediment bioassays to metal-contaminated areas in Wisconsin, Michigan and California. Bioassay methods have also been used to determine toxicity in freshwater sediments experimentally spiked with copper (Cairns et al., 1984) and cadmium (Schuytema et al., 1984).

A commonly used freshwater sediment bioassay method was developed by Prater and Anderson (Prater and Anderson, 1977a,b; Prater and Hoke, 1978), and modified by others (LeBlanc and Surprenant, 1985; Malueg et al., 1983, 1984 a and b; Schuytema et al., 1984). In this system burrowing or epibenthic organisms (usually the insects Chironomus or Hexagenia, the amphipods Hyalella or Gammarus or the isopod Asellus) are exposed to test sediment, while swimming organisms (the cladoceran Daphnia or the fathead minnow Pimephales) are exposed to water recirculating above the sediment. Malueg et al. (1983) reported that the presence of Hexagenia in the sediment in this system increased the toxicity of the water to Daphnia.

In solid phase static bioassays test organisms are exposed to sediment with overlying water in a beaker, and may be present in the sediment or water or both. Cairns et al. (1984) employed acute static bioassays to test toxicity of copper-spiked sediments to Daphnia, Chironomus, Hyalella, and Gammarus. Wentsel et al. (1977,1978) used Chironomus larval growth and survival and adult emergence as response criteria in solid

phase static bioassays with naturally contaminated sediments.

A variety of procedures have been developed to test biological effects of sediment extracts or elutriates. A sediment elutriate test in which Daphnia are exposed to water extracts of sediments in 250 ml beakers was developed as a modification of the EPA/COE (1977) recommended procedure for evaluating the toxicity of dredged sediments disposed in open waters (Jones and Lee, 1978; Lee et al., 1978). Chapman and Brinkhurst (1984) and Chapman et al. (1982a,b) described test procedures using marine and freshwater oligochaete respiration response to toxicants in water, and have applied these methods to marine sediment extracts (Chapman et al., 1982c). Another method, utilizing mitotic abnormalities in cultured rainbow trout cells in response to marine sediment extracts (Chapman et al.,1982c; Landolt and Kocan, 1984), has recently proven to be sensitive with freshwater sediment extracts as well (Chapman, 1985). Samoiloff et al. (1983) described bioassay methods using bacterial mutagenicity and inhibition and nematode survival, growth and mutagenicity in response to sediment extract fractions from a lake in Saskatchewan.

Nebeker et al. (1984) used a series of three acute and four chronic sediment bioassay procedures. Acute procedures tested the toxicity of sediment elutriates to Daphnia or of sediment with overlying water to Daphnia, Hyalella, Chironomus or Gammarus in beakers. Results using these methods compared favorably with those using the Prater-Anderson system

with Daphnia and Hexagenia. Chronic methods used Daphnia and Hyalella life cycle data and Chironomus larval survival and growth and adult emergence as response criteria. Chapman (1985) reported that Daphnia are very sensitive indicators of toxicity in both the acute and life cycle tests, but that the other organisms used by Nebeker et al. (1984) were less sensitive.

In a series of partial life cycle tests using static and flow through systems, Adams et al. (1985) demonstrated that the key route of exposure of a persistent toxic chemical (Kepone) in sediment to the midge, Chironomus tentans, was via the interstitial water. The results provide insight into the mechanism of action of toxicants in sediments to benthic organisms.

2.3. Great Lakes

In the Great Lakes, sediment toxicity testing has been primarily applied to assessing the impact of dredged sediment disposal. The Prater-Anderson apparatus has frequently been used for testing sediments with recirculating water and the organisms Daphnia, Hexagenia, Asellus, and Pimephales. Although some positive correlations were found, results of bioassays using this system did not correlate well with most individual chemical parameters examined in sediments from Duluth and Superior Harbors (Prater and Anderson, 1977b), or from five Lake Michigan

harbors (Hoke and Prater, 1980; Laskowski-Hoke and Prater, 1984; Prater and Hoke, 1979,1980). Using a modification of the Prater-Anderson procedure with sediment from the Keweenaw Waterway, Lake Superior, Malueg et al. (1984a) did find positive correlations among sediment bioassay results, copper content in the sediments and in the overlying water in the bioassay chambers, and major disruptions in the benthic macroinvertebrate community structure.

Gannon and Beeton (1969,1971) surveyed nine Great Lakes harbor sediments for toxicity using a 48-60 hour acute static bioassay system and a 24-48 hour sediment selectivity procedure. Response to polluted sediments was observed using both test procedures with the amphipod Pontoporeia and the opossum shrimp Mysis. Oxberry et al. (1978), using long-term (up to one year) static tests with Pontoporeia and Mysis tested the toxicity of taconite tailings in Lake Superior to benthic organisms. Bahnick et al. (1980) performed extensive series of 96 hour bioassays using Hexagenia, Daphnia and Pontoporeia to test sediments from Duluth-Superior Harbor and Lake Superior. They tested sediment with overlying water, interstitial water and sediment elutriate water and demonstrated good survival with all animals in the test systems in controls and in most harbor sediments tested.

3. THE RHEPOXYNIUS SEDIMENT BIOASSAY

3.1. Method

The Rhepoxynius sediment bioassay is a revision of the multispecies sediment bioassay method developed in our laboratory for dredged material evaluation (Swartz et al, 1979). The present method is a ten-day acute static test with solid phase sediment and overlying water in 1 liter aerated beakers. (See Swartz et al., 1985a for a detailed method description.) The test animal, Rhepoxynius abronius, is a 3-5 mm burrowing amphipod that does not make tubes and does not normally extend body parts from the sediment. It inhabits primarily the upper 2 cm of sediment and leaves the sediment infrequently, although emergence and swimming behavior have been observed under some conditions, as in the presence of infaunal predators (Ambrose, 1984). R. abronius has been found from the lower intertidal to depths of 274 m offshore, and is present year round at our collection site at densities of 150-750/m^2 (Barnard and Barnard, 1982; Kemp et al., 1985).

The bioassay chamber is a standard 1 liter beaker containing 2 cm of test sediment and filtered 25-28 ppt seawater up to the 950 ml mark on the beakers. Twenty amphipods are distributed to each beaker and allowed to bury. Beakers are covered with watch glasses, aerated, and held at 15° C under constant illumination for ten days. Emergence of the amphipods from the

sediment is correlated with toxicity and is monitored daily during the course of the bioassay.

After ten days the sediment in the beakers is sieved and the numbers of live and dead amphipods are counted. Live amphipods are transferred to clean sediment and the numbers that are able to rebury within one hour are counted. Survival and reburial data for test sediment are statistically compared with control data. Control survival almost always exceeds 95%. Failure of surviving amphipods to rebury in clean sediment is directly related to degree of sediment contamination and amphipod mortality within a treatment, and is a sublethal indicator of sediment toxicity.

The Rhepoxynius bioassay has been applied to both field collected sediments and to uncontaminated sediments spiked in the laboratory with specific contaminants. Field sediments are collected with a core or grab to minimize disruption of the sediments, and generally only the upper 2 cm of sediment are used in a bioassay. Test sediments are collected within a few days of the initiation of a bioassay if possible and are stored at 4^{o} C. Freezing or longer storage has been shown to change the toxicity of the sediment (Swartz et al., unpubl.). Sediments are thoroughly mixed before they are put into bioassay beakers, but field sediments are not sieved. Uncontaminated sediment from the amphipod collection site can be spiked with individual or combined contaminants. After spiking, sediment is mixed in a rolling mill to evenly distribute the

contaminant (Ditsworth et al., ms. in prep.).

The static bioassay system represents a "worst case" situation in which contaminants remain within the system and are available to the test organism. The amphipods have been shown to be tolerant of a wide range of physical and chemical sediment parameters such as grain size and organic enrichment, but are appropriately sensitive to toxicants (Swartz et al., 1984, 1985a).

3.2. Field Validation

The purpose of a field validation is to test the ecological relevance of a laboratory bioassay technique in terms of correspondence between bioassay results and pollution effects in real ecosystems. Swartz et al. (1982) found that in Commencement Bay, Washington, amphipods were abundant and diverse in areas of low toxicity to Rhepoxynius, but amphipods were rare and phoxocephalids were absent in the industrialized waterways, where sediments were often highly toxic bioassays. Swartz et al. (1985b) also found that transect stations closer to a large sewage outfall exhibited decreased survival of Rhepoxynius in bioassays. This was related to decreased biomass, density, species richness and dominance in the macrobenthic community, and increased concentrations of chemical contaminants relative to stations further from the outfall where Rhepoxynius survival was high. Other authors have also used the Rhepoxynius bioassay and

reported similar sensitivity to contaminated sediments, and correspondence between the Rhepoxynius and other sediment bioassays, sediment chemistry, and bottom fish histopathological abnormalities (Chapman, 1985; Chapman et al., 1982c; Mearnes et al., 1985; Quinlan et al., 1985).

3.3. Laboratory Applications

The bioassay can be applied to test the toxicity of individual chemicals added to unpolluted sediments, or to determine the toxicological effects of interactions among chemicals in artificially spiked sediments. Swartz et al. (1985c) determined the LC50 of cadmium in sediments and water in 96 hour and ten day bioassays with Rhepoxynius. They found that sewage sludge or the fine fraction of natural sediment particulates added to cadmium spiked sediment reduced the toxicity, as the added components apparently adsorbed some of the cadmium. Studies on individual chemicals, however, provide only a part of the information necessary to evaluate the biological effects of complex mixtures of chemicals deposited in sediments. Toxicological interactions among chemicals may be additive, synergistic or independent, and laboratory studies may be applied to determine or predict the effects of particular combinations of chemicals.

4. PROBLEMS WITH SEDIMENT BIOASSAYS

Sediment bioassays are more complex than water bioassays because of the variable properties of the sediment itself. Sediment handling during sampling and bioassay setup may change the chemical properties of the intact sediment through oxidation, mixing of layers, removal of interstitial water, and disruption of sediment structure. Storage of sediments has been found to change their toxicity (Swartz et al., unpublished) and freezing should be avoided. Chemicals sorbed on sediment particles are less bioavailable than dissolved substances, and bioavailability changes with factors such as organic content and oxidation state. Toxicity of sediments to some organisms may depend on sediment properties other than chemical contamination, such as grain size and in the case of Rhepoxynius, interstitial water salinity. Monitoring of toxicant effects on infaunal test organisms is difficult as the animals are not normally seen until the bioassay is terminated, and the presence of predators or other organisms in the test sediment could affect results. Finally, when toxicity is found, it is difficult to determine whether the observed effects are due to toxicants associated with the sediment particles ingested by the test organism or to chemicals dissolved in interstitial water.

5. APPLICATIONS OF SEDIMENT BIOASSAYS

Sediment toxicity bioassays may be applied in various ways in field and laboratory research as well as in regulatory programs. The spatial or temporal distribution of sediment toxicity can be investigated or monitored in relation to pollution gradients or potential sources. Sediment bioassays reveal unsuspected problem areas which should be analyzed in more detail using sediment chemical analyses or other ecological monitoring techniques. Bioassays can be used to rank problem areas in terms of toxicity to benthic organisms, to establish priorities and to determine the limits of small scale problem areas for possible cleanup. Sediment bioassays can be a part of the assessment of toxicity in sediments subject to regulatory decisions, and are being used successfully by the Corps of Engineers in its dredged material permit program. Bioassays could be used to establish sediment quality criteria, regulatory limits and guidelines for disposal permit programs for complex wastes such as sewage sludge and industrial effluents. In the laboratory, chemicals can be added to uncontaminated sediments to determine toxicity to benthic organisms, and to identify which chemicals or groups of chemicals may be causing biological effects observed in the field (Long, 1983).

6. RECOMMENDATIONS FOR RESEARCH

The following recommendations are made for research in the use of sediment toxicity bioassays in the Great Lakes and other areas of concern:

a. Develop biological testing methods for assessment of contamination in benthic ecosystems. No single technique can meet all requirements for sediment testing, and various methods should be developed and results compared. Bioassays using the amphipod Pontoporeia have been employed by Bahnick et al. (1980), Gannon and Beeton (1969,1971) and Oxberry et al. (1978). Pontoporeia is an abundant indigenous resident of the Great Lakes, ecologically important, easy to maintain in laboratory and test systems, and tolerant of a wide range of natural sediment characteristics, but is sensitive to sediment contaminants (Alley, 1968; Kraft, 1979; Marzolf, 1965; Mozley and Howmiller, 1977; Nalepa and Thomas, 1976; Smith, 1972). These are important criteria as suggested by Buikema et al. (1982) in selection of species for bioassays, and it is proposed that bioassay methods may be developed using this species. Possibly a system such as that used in our laboratory for Rhepoxynius could be developed for Pontoporeia. Field validation should be an important part of the development of any new bioassay method.

b. Other ecological assessment techniques should be used in combination with bioassays to define areas of concern and to determine the current extent of pollution problem areas. A

stepwise multidisciplinary approach for the assessment of pollution in coastal areas has been devised and successfully applied in Puget Sound (Chapman and Long, 1983; Long, 1983), and a similar approach is recommended for the Great Lakes and other problem areas.

c. Perform chemical analyses on contaminated sediments revealed as toxic by bioassay results. Correlation of sediment toxicity with single contaminants is rare, as toxicity is most often due to a complex mixture of chemicals, but where causative agents can be identified, it may be possible to locate and eliminate sources of toxicity. Statistical correlation may be increased and costs reduced if replicate samples for bioassays and chemical analysis or benthic community structure are taken simultaneously from the same grab or the same station.

d. Determine effects of specific toxic substances as deposited in sediments on resident biota. Sedimentological properties such as organic content and particle size can change the bioavailability of sediment toxicants, and more research is needed in determining the biological and ecological effects of individual and groups of chemicals deposited in sediments. Laboratory studies may be employed to assess effects of individual chemicals, the interaction of toxic substances in combination, and the effects of sediment partitioning and organic content on test organisms.

7. CONCLUSIONS

Sediment bioassays alone do not provide all of the answers in assessment of sediment pollution effects on benthic ecosystems, but they can be used as a cost-effective tool in initial sediment toxicity surveys. They can be used in combination with other ecological assessment techniques in more detailed evaluations of sediment contamination, or in laboratory studies of biological effects of toxic substances. Sediment bioassays are being used successfully in regulatory programs. Field validation is an important part of the development of any new bioassay method, and more research is needed in the Great Lakes and in other areas on the ecological consequences of persistent toxic substances in sediments.

ACKNOWLEDGMENTS

Appreciation is extended to Dr. Paul Kemp and Dr. Alan Nebeker for reviewing the manuscript.

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